Minimal Residual Disease (MRD) as a Surrogate Endpoint in ALL
FDA Workshop, 18 April 2012, Silver Spring, MD

Minimal residual disease in acute lymphoblastic leukemia

Technical, economical, and validation – QC considerations for multicenter MRD assessment

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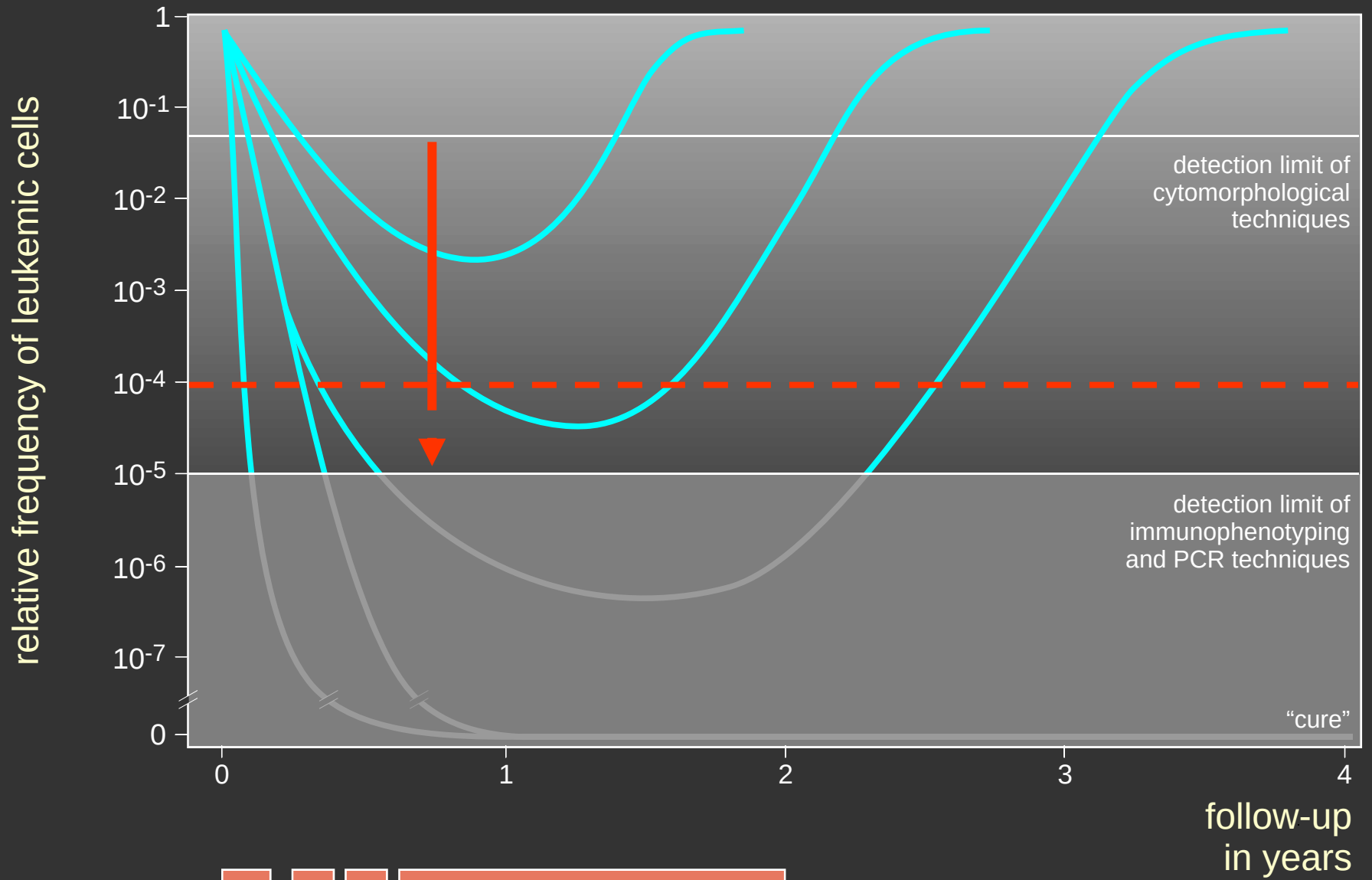
Detection of minimal residual disease (MRD) in ALL



DCLSG ALL-8 | I | C | II | maintenance Rx

Dept. of Immunology, Erasmus MC, Rotterdam

Detection of minimal residual disease in acute leukemia

Technique	Applicability	Detection limit	Remark
Flow cytometry (4 to 6 colors)	BCP-ALL: 85% T-ALL: 90% AML: 60-70%	(10^{-3}-) 10^{-4}	Fast, but variable sensitivity because of similarities between normal (regenerating) cells and malignant cells
PCR of Ig/TCR genes	BCP-ALL: 95% T-ALL: 95% AML: 10-15%	10^{-4}-10^{-5}	Time consuming and relatively expensive (junctional region sequencing), but applicable in ≥ 95% of lymphoid malignancies
PCR of fusion transcripts and mutations	BCP-ALL: 40% T-ALL: 25% AML: 25-40%	10^{-4}-10^{-6}	Limited applicability in ALL, but potentially useful in specific subgroups, e.g. BCR-ABL cases in specific protocols

MRD diagnostics in ALL

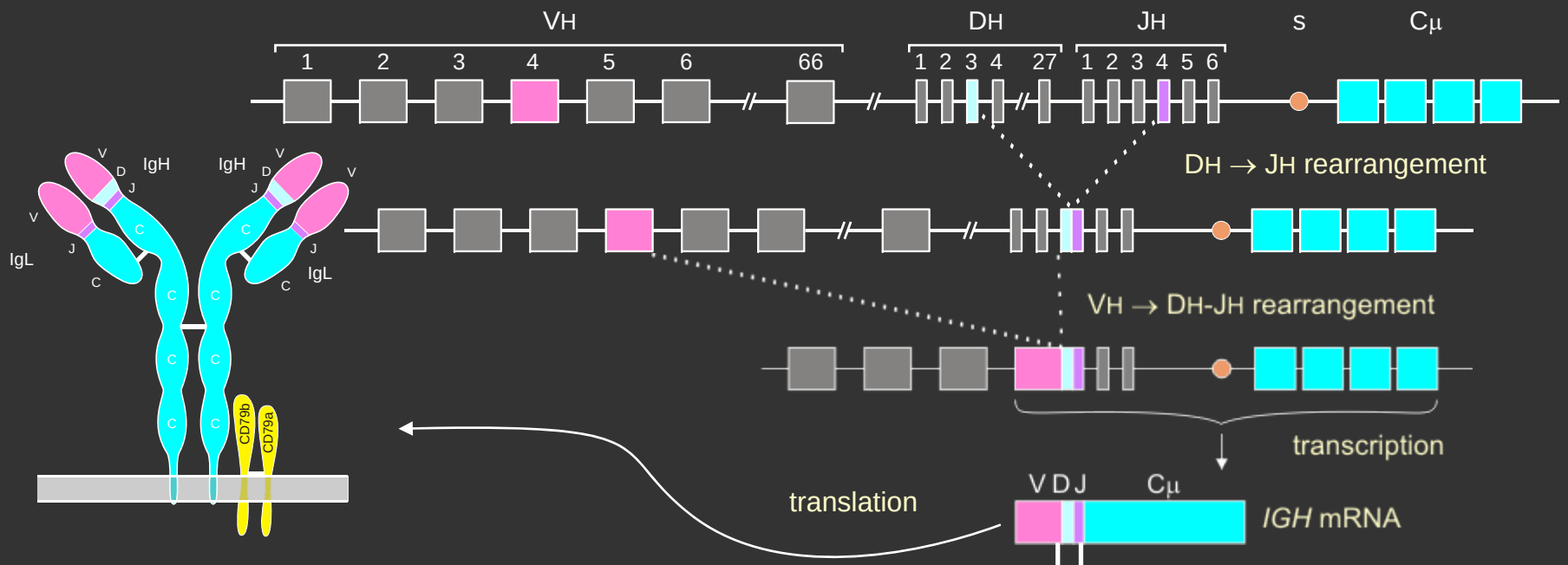
From research tool to surrogate endpoint in ALL treatment

1. Definitions of MRD remission, MRD recurrence, relapse
2. When should MRD be measured and which sensitivity
3. Evaluation of:
   - Induction Treatment
   - Continuous Monitoring
   - Treatment blocks (new drugs)

   } Dependent on disease category and treatment protocol
4. Comparison between MRD studies: time points and quantitative range & sensitivity
5. MRD techniques: Ig/TCR PCR and/or Flow Cytometry
6. Standardization and Quality Control

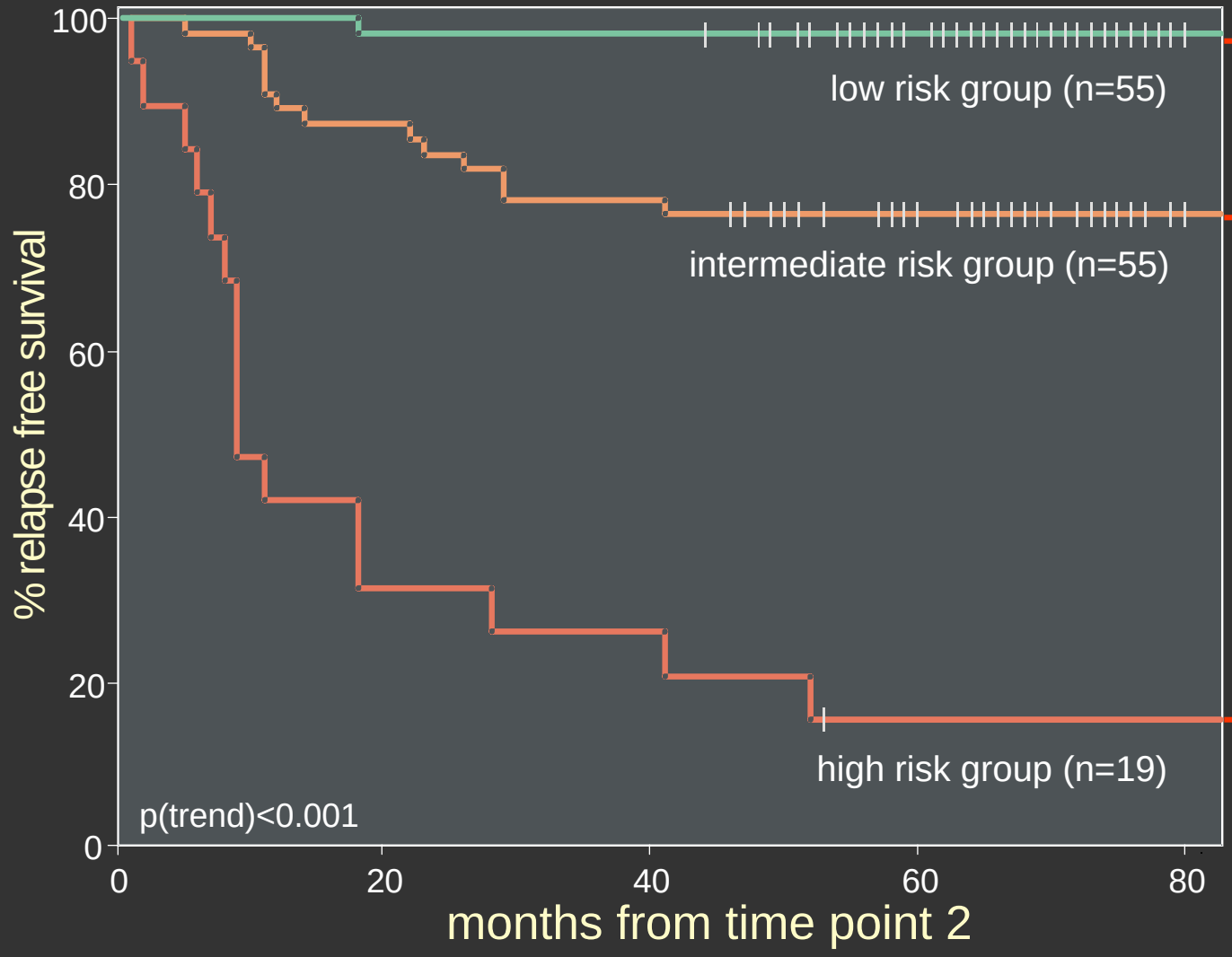
Collaborative networks on standardization & quality control

From Ig gene to Ig molecule



VH3-21 (germline)	insertion	DH3-3 (germline)	insertion	JH4-1 (germline)
TGTATTACTGTGCGAGA		GTATTACGATTTTTGGAGTGGTTATTATACC		ACTACTTTGACTACT
TGTATTACTGT	AGGC	CGATTTTTGGAGTGGTTATTATA	GTCCA	TGACTACT
TGTATTACTGTGCG	TATCCGGA	TTACGATTTTTGGAGTGGTTATTATAC	CGATCG	CTTTGACTACT
TGTATTACTGTGC	CCGGACTG	TTTTGGAGTGGTTATTATACC	GGT	ACTACTTTGACTACT
TGTATTACTGTGCGAG	CTGAGTC	TATTACGATTTTTGGAGTGGTTAT	CGTAGCGTA	TTTGACTACT
TGTATTACTG	ACATCGA	CGATTTTTGGAGTGGTTATTATA	CGTAG	ACTTTGACTACT
TGTATTACTGTGCG	CGT	TACGATTTTTGGAGTGGTTATTAT	GGCTAAGG	TGACTACT
TGTATTACTGTGCGA	CCGG	TTACGATTTTTGGAGTGGTTATTATACC	CGGAGC	TACTTTGACTACT

Relapse free survival in I-BFM-SG study according to the combined MRD information at time points 1 and 2 (n=129)



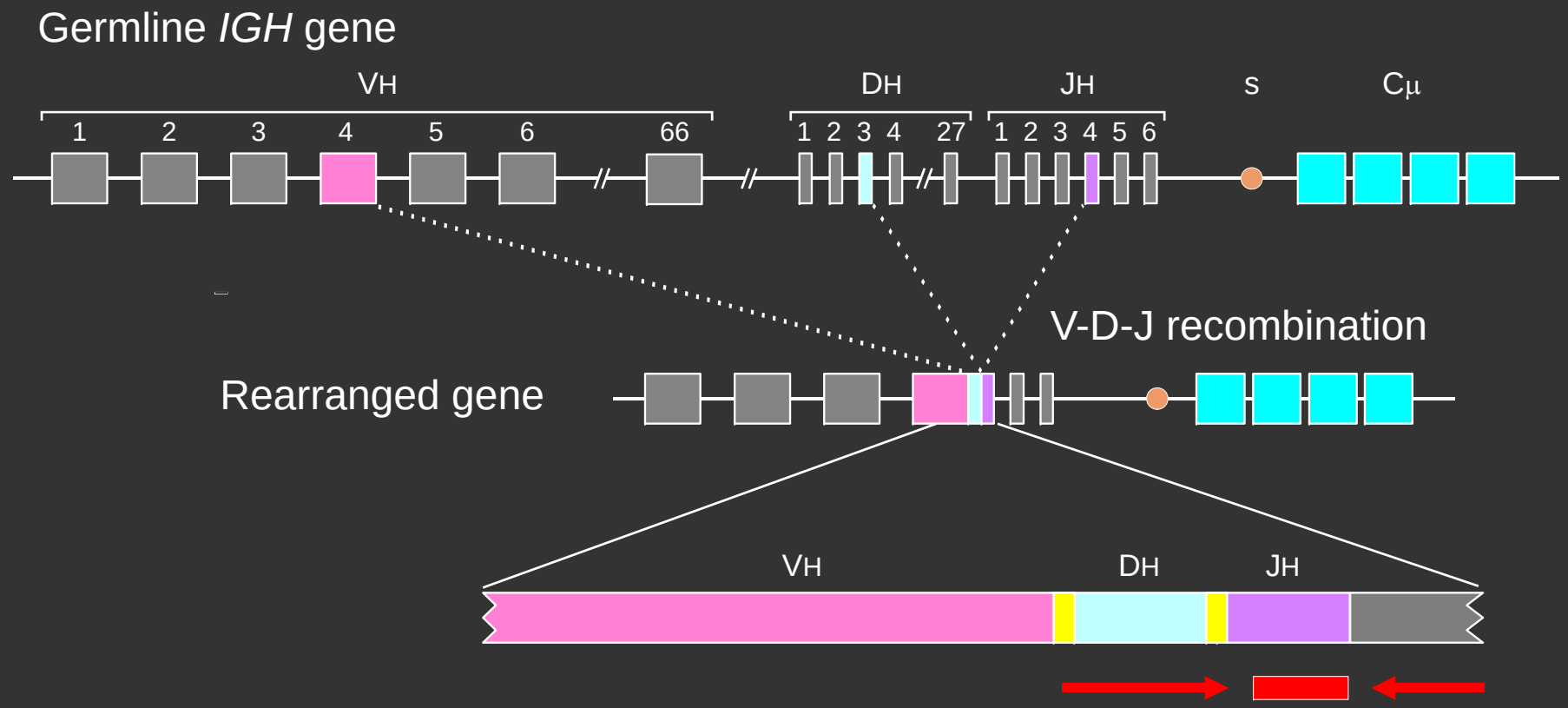
43% of patients
4% of relapses

43% of patients
42% of relapses

14% of patients
54% of relapses

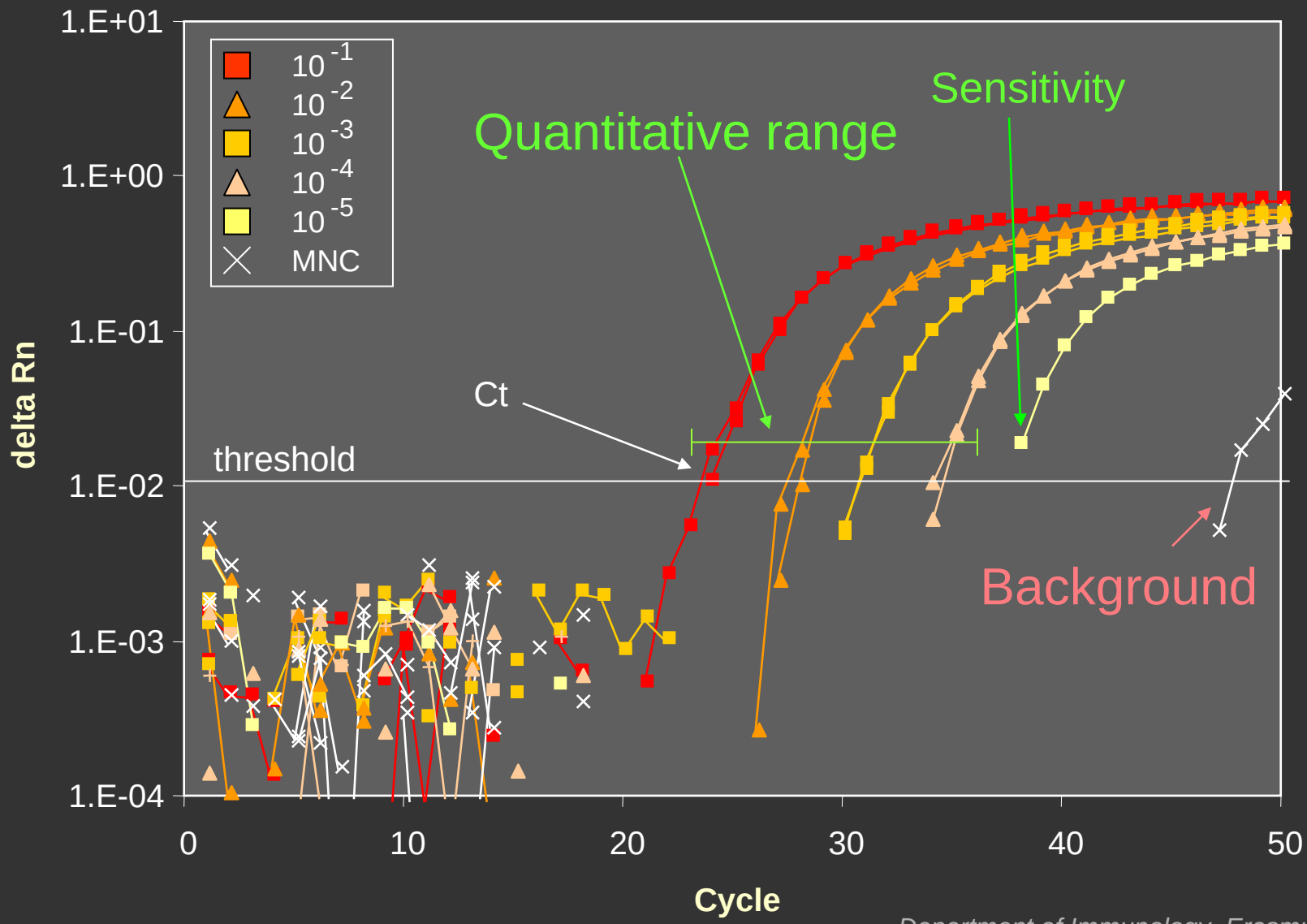
I-BFM-SG Report, J.J.M. van Dongen et al, Lancet 1998;352:1731-1738

PCR analysis of Ig/TCR genes



→ High levels of standardization required

Guidelines for RQ-PCR analysis of TCR/Ig gene rearrangements

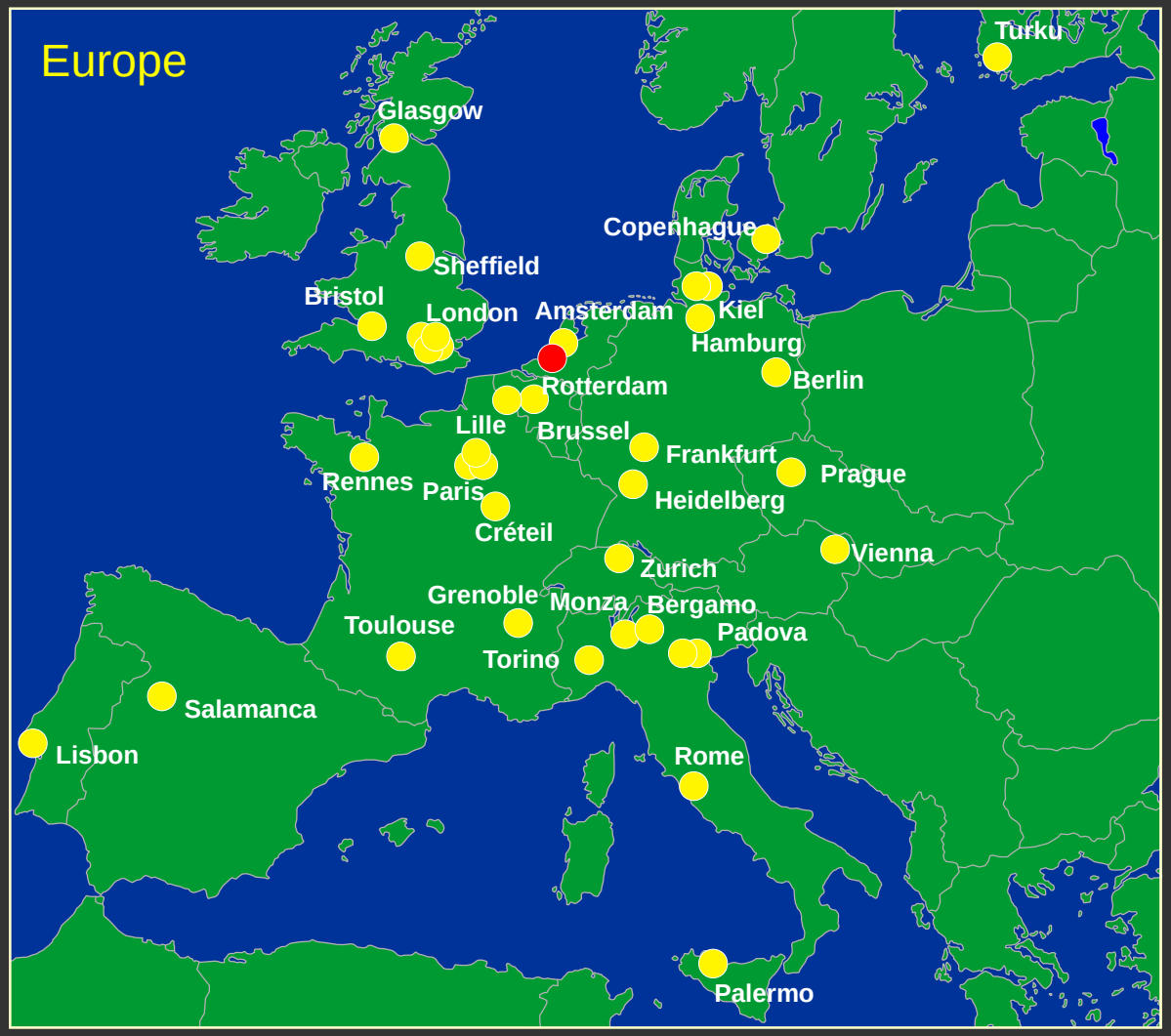


Department of Immunology, Erasmus MC, Rotterdam

European Study Group on MRD detection

Chairman: J.J.M. van Dongen

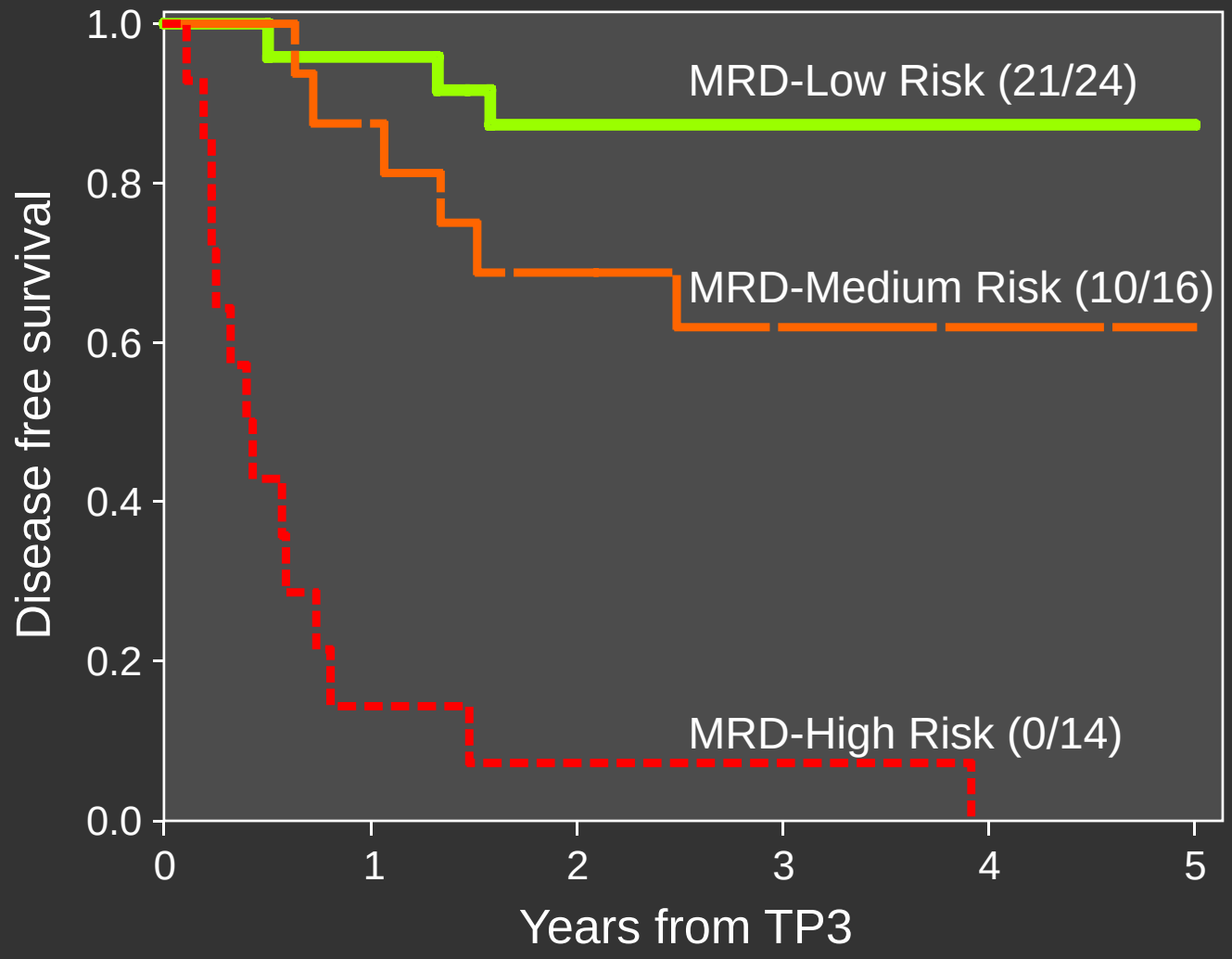
www.EuroMRD.org



43 laboratories in 18 countries

Supported by Leukaemia & Lymphoma Research, LeukemiaNet, and EuroClonality

MRD diagnostics in infant ALL: Interfant-99 protocol



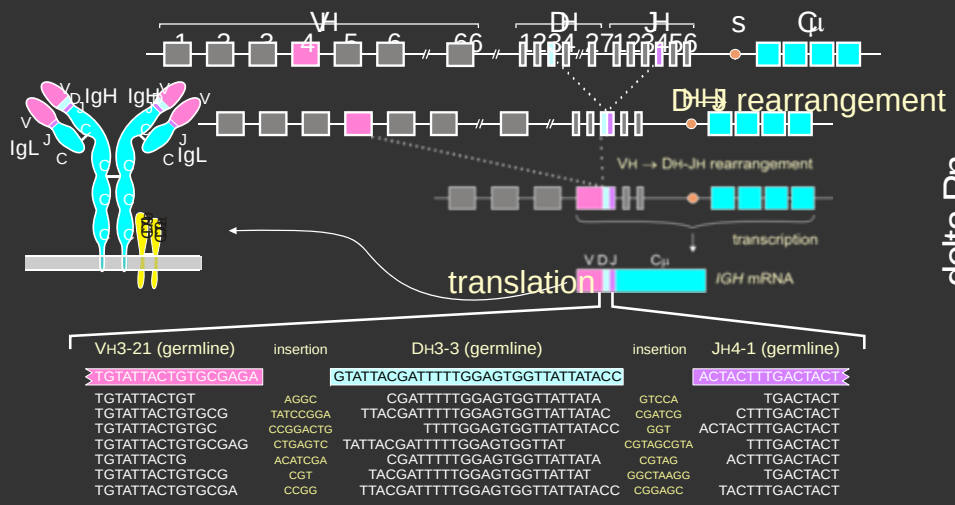
44% of patients
13% of relapses

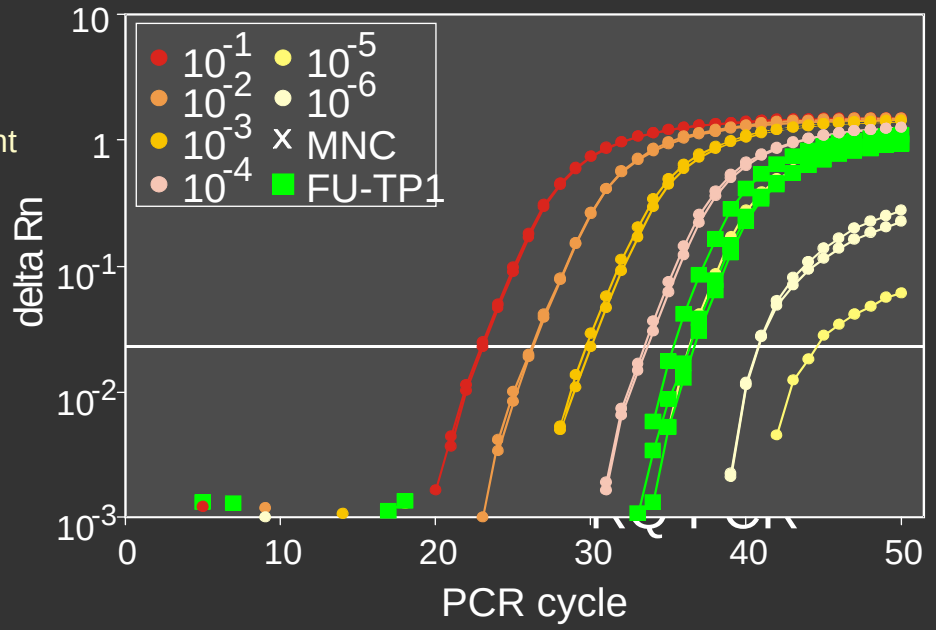
30% of patients
26% of relapses

26% of patients
61% of relapses

Van der Velden et al., Leukemia 2009; 23: 1073-1079

Current MRD technique in lymphoid malignancies





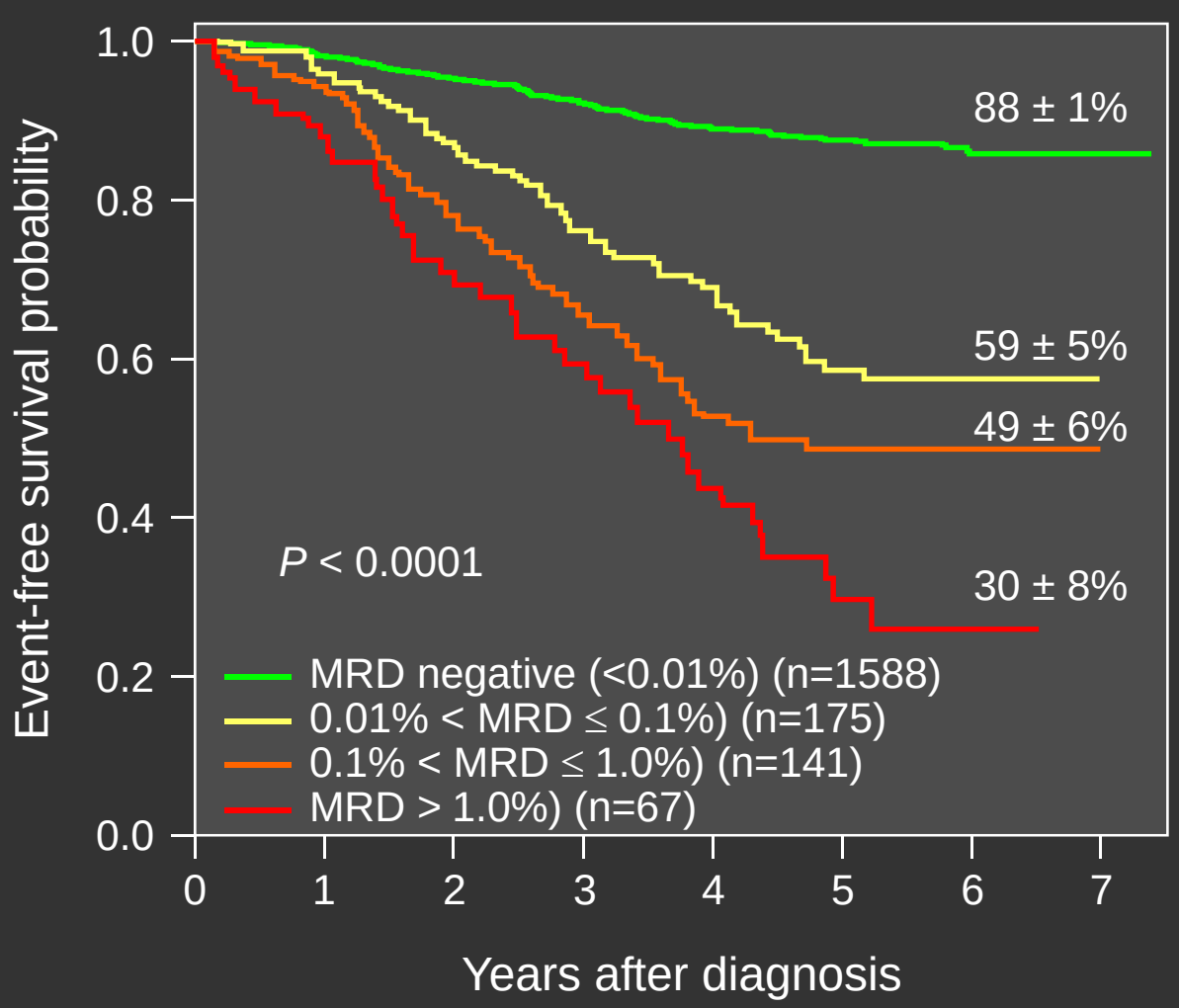
Disadvantages of Ig/TCR-based MRD-PCR techniques:

labor intensive (junctional regions per patient);

- require specialized laboratories;
- time consuming (target identification: 4 to 6 weeks)

→ Faster technique needed: 8-color flow cytometry ?

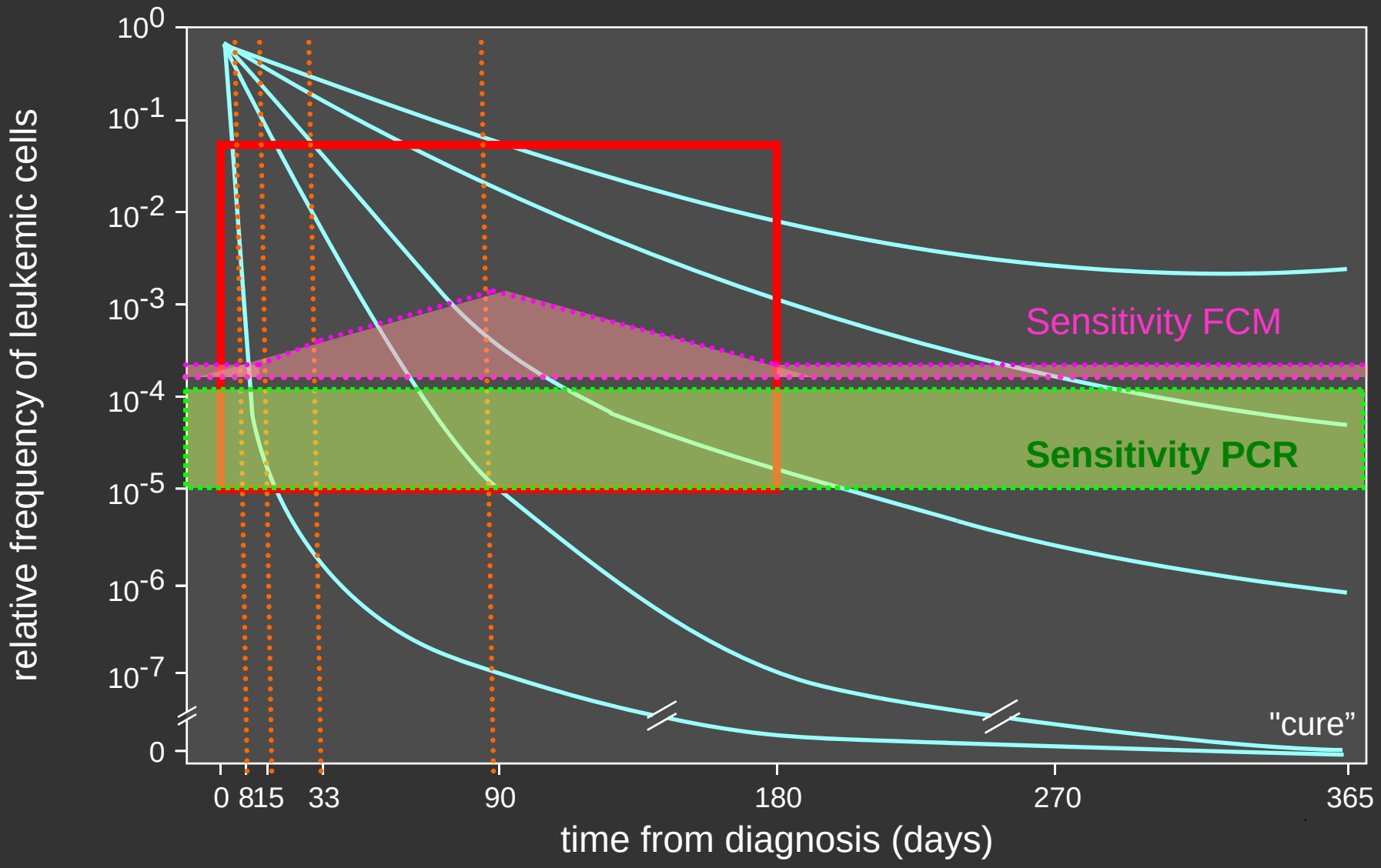
EFS of MRD-based risk groups (FCM at day 29) in COG protocol



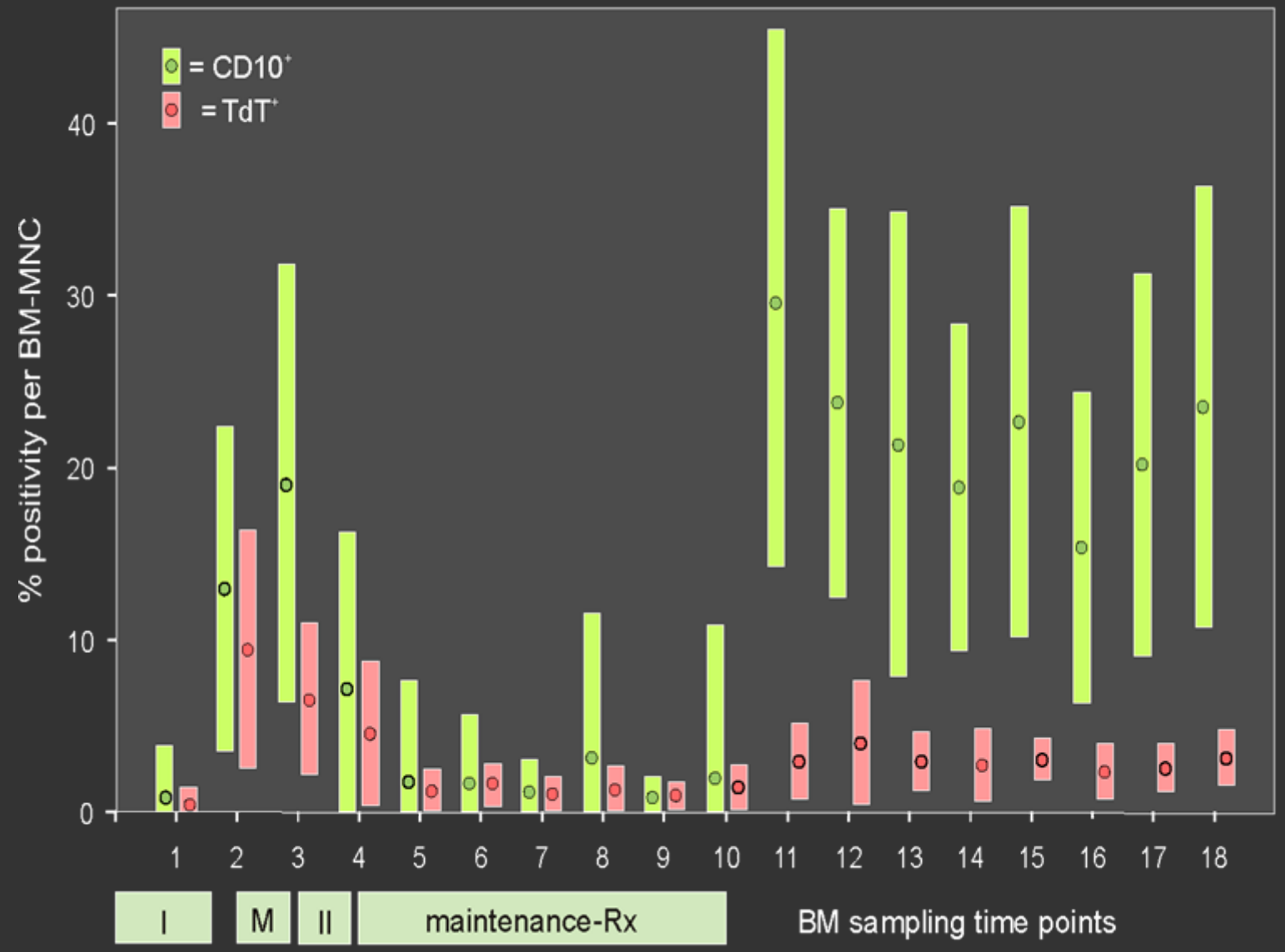
80% of patients
51% of relapses

Borowitz et al., Blood 2008; 111: 5477-5485.

MRD window, time points, MRD techniques and QR & sensitivity

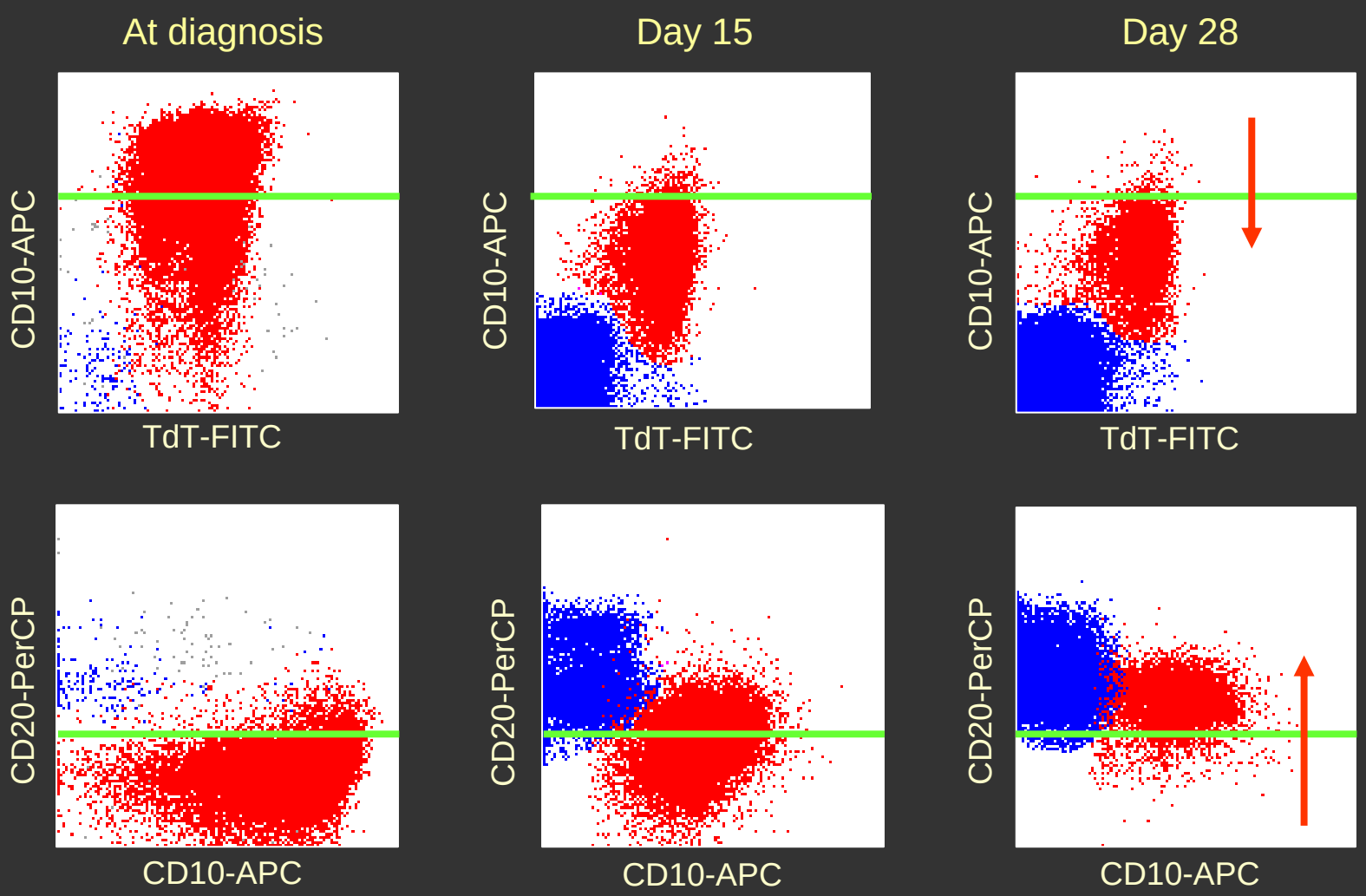


Precursor-B-cells in BM during ALL treatment



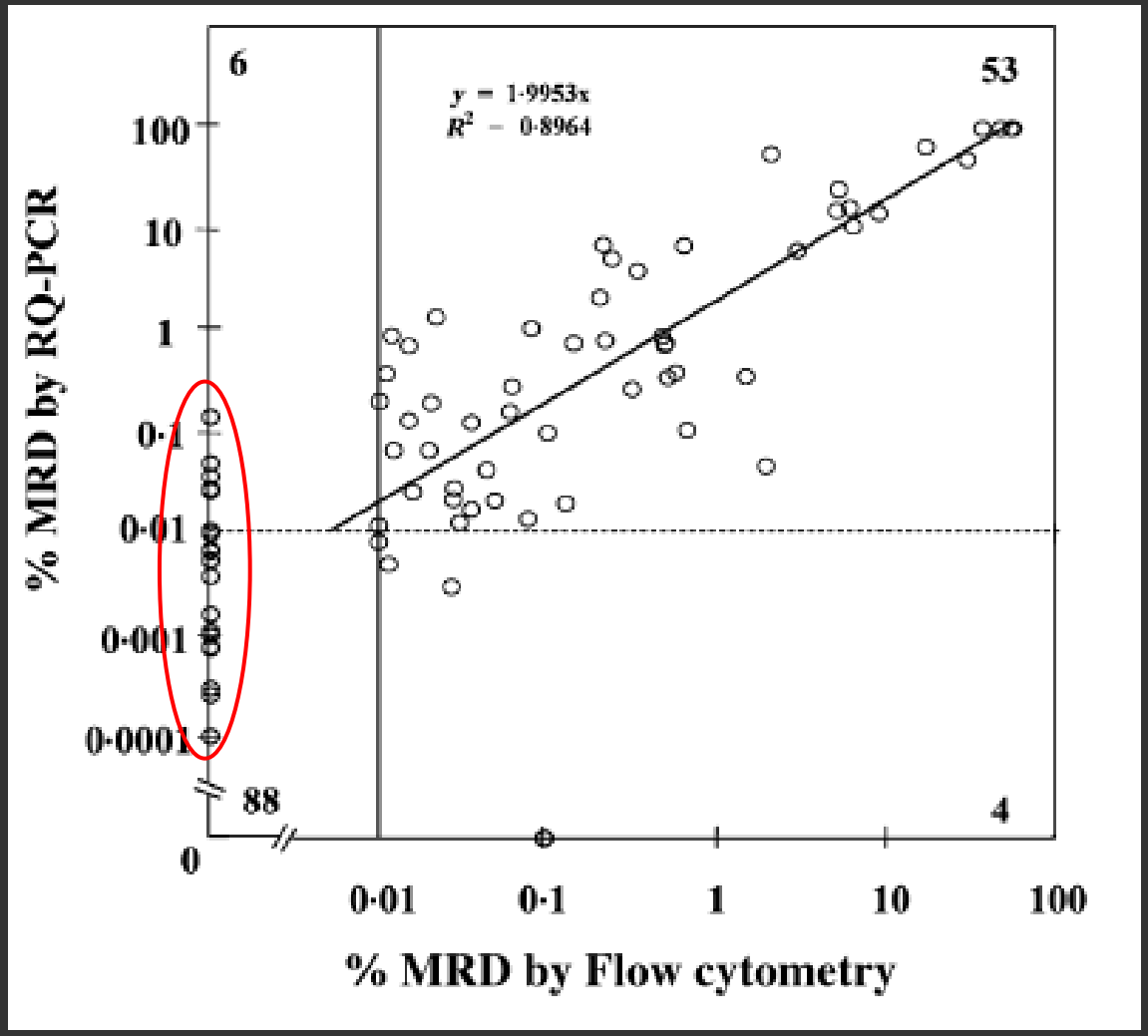
Van Wering et al., Brit J Haematol 2000;110:139-146

Therapy-induced immunophenotypic shifts



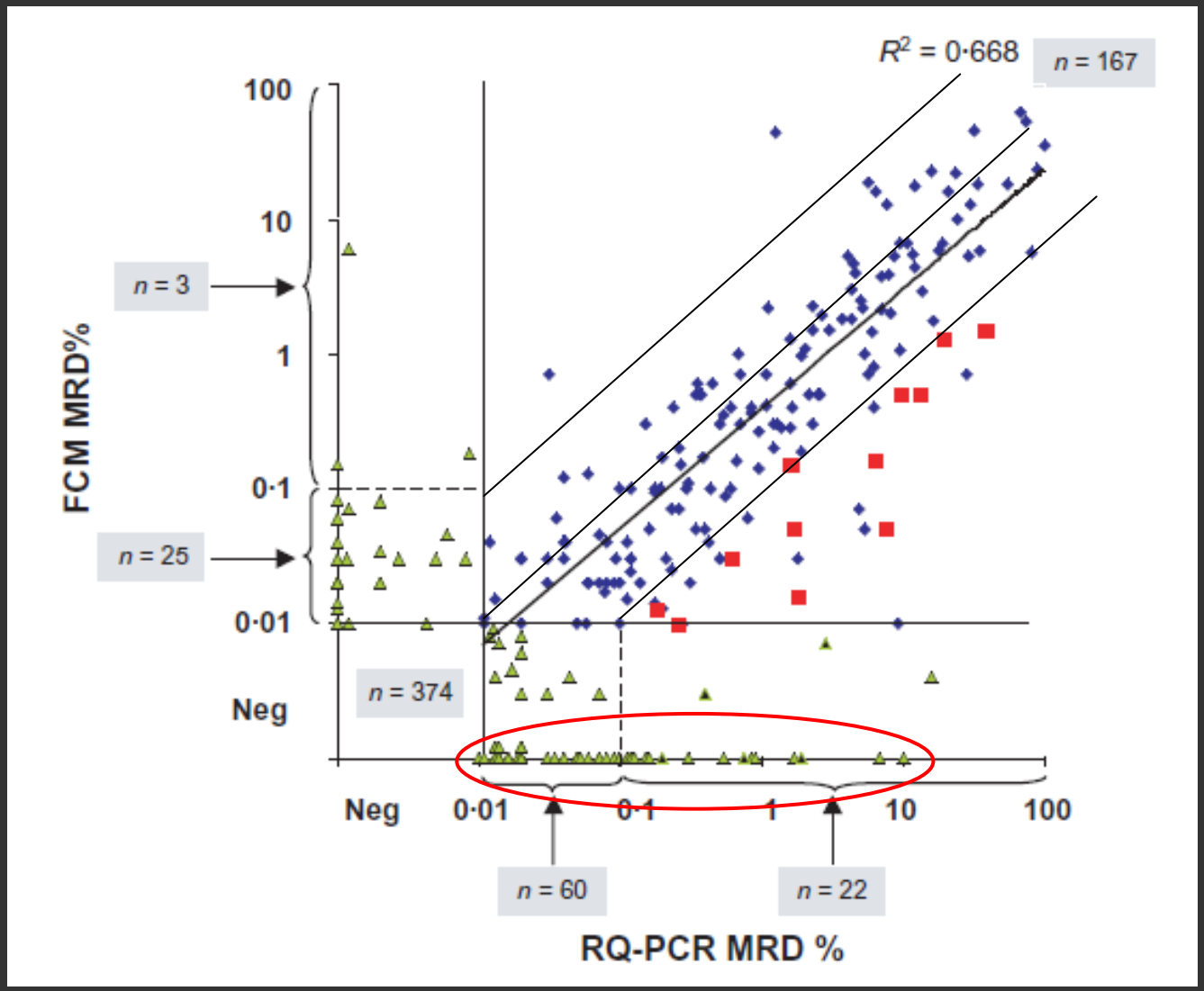
Van der Sluijs et al, LEUKEMIA 2005; 19: 1845-1847

RQ-PCR and flowcytometric MRD in childhood ALL



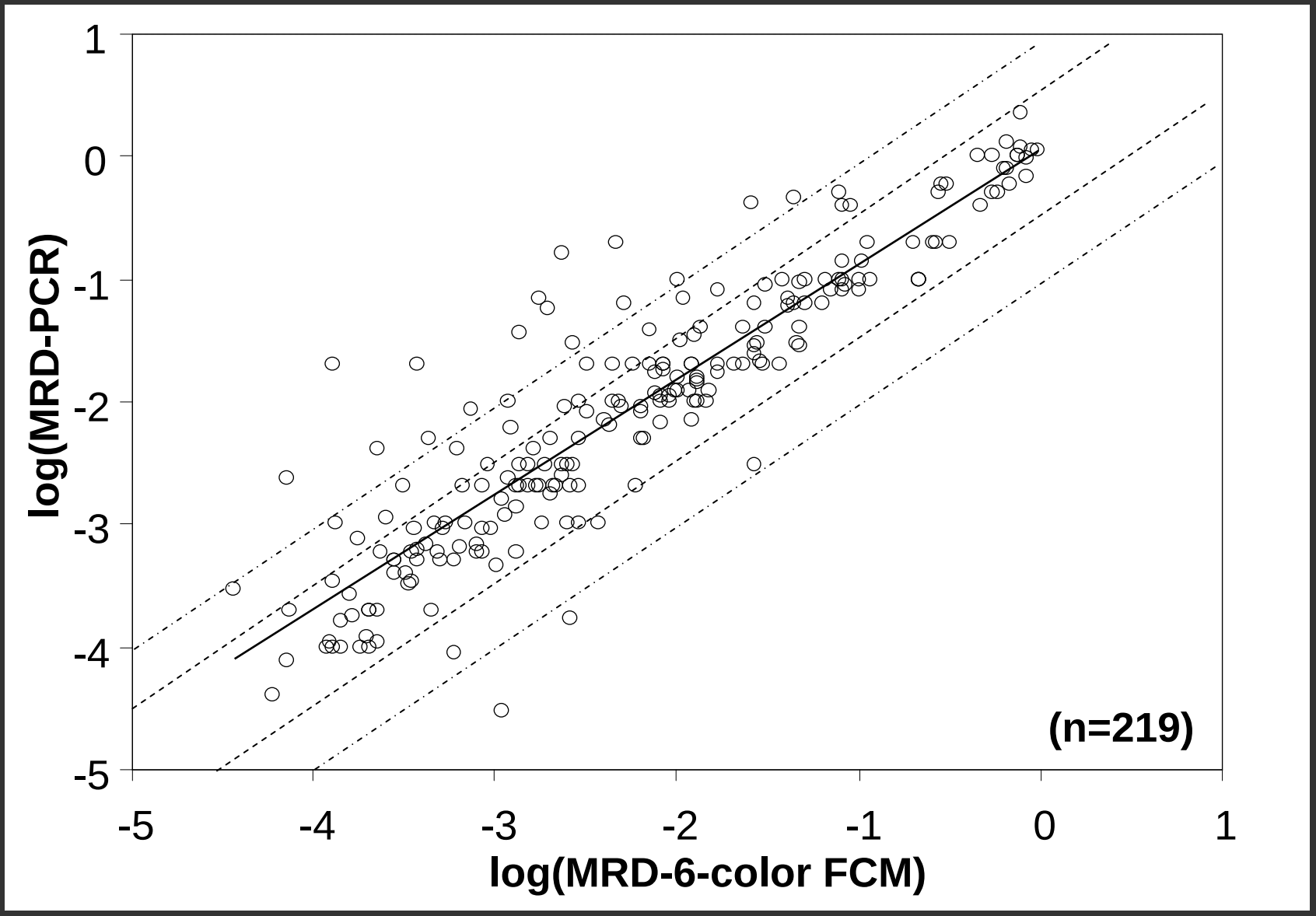
Ryan J., et al. MRD detection in childhood ALL patients at multiple time-points reveals high levels of concordance between molecular and immunophenotypic approaches. Br J Haematol 2008 144: 107-115

RQ-PCR and flowcytometric MRD in childhood ALL



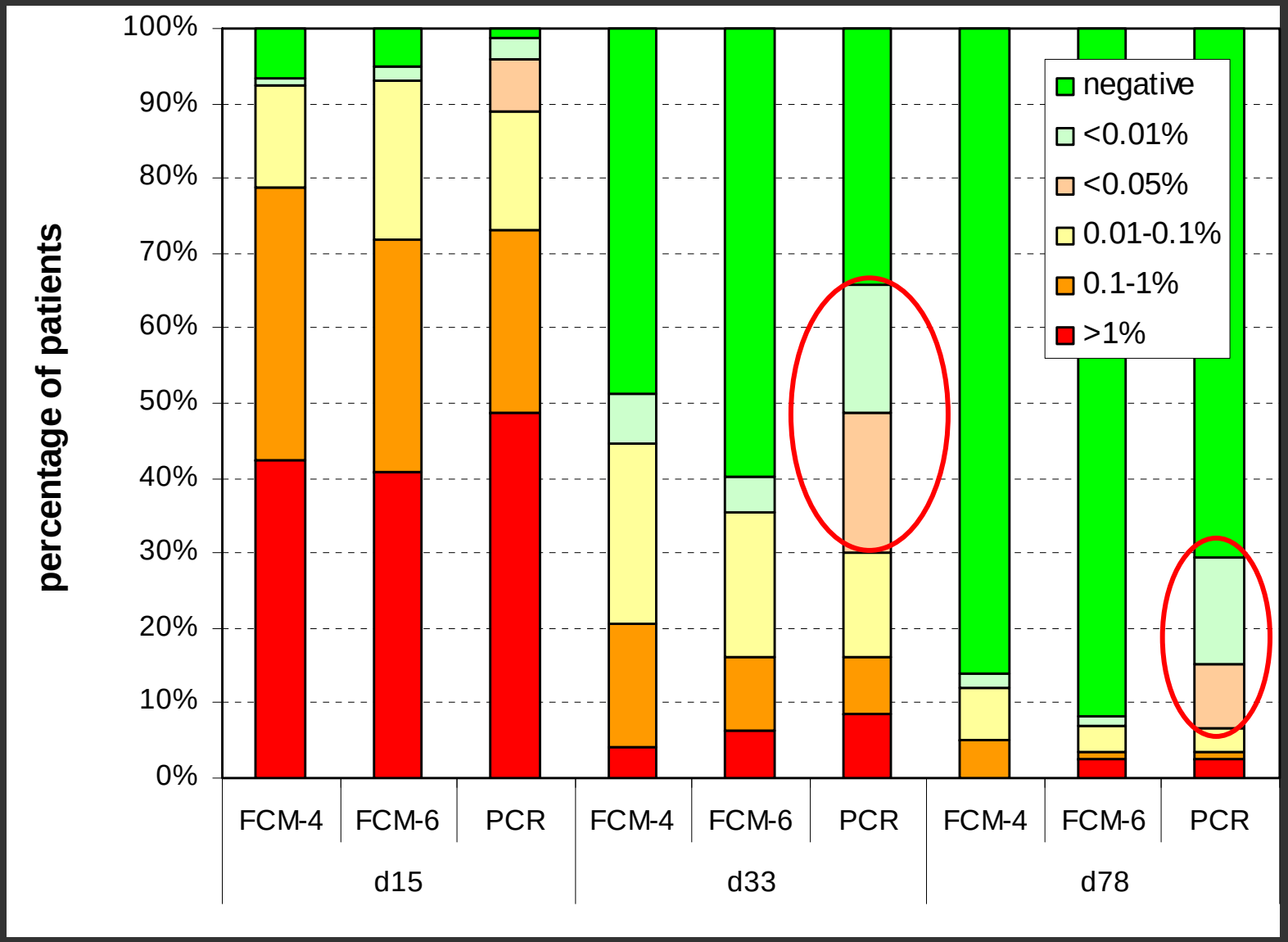
Thörn, I. et al. MRD assessment in childhood ALL: a Swedish multi-centre study comparing real-time polymerase chain reaction and multicolour flow cytometry. Br J Haematol 2011 152: 743-753

RQ-PCR and flowcytometric MRD in childhood ALL (only positive data in quantitative range)



V.H.J. van der Velden et al., Unpublished results

RQ-PCR and 4-color/6-color flow cytometric MRD in childhood ALL



V.H.J. van der Velden et al., Unpublished results

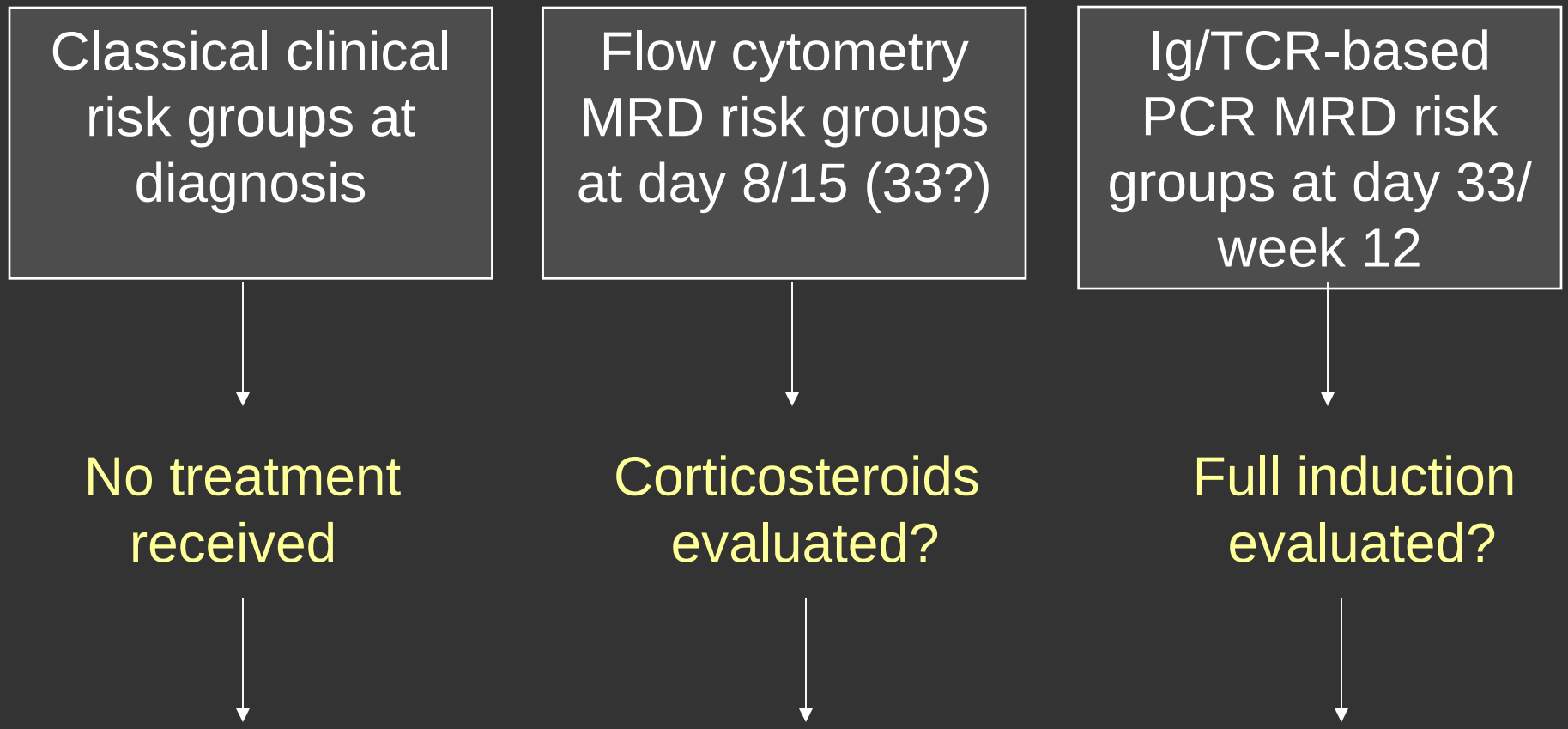
MRD-based risk groups (day 33 and day 78) in 171 patients of the DCOG-ALL10 protocol

		RQ-PCR (Ig/TCR) based risk groups			
		HR (6%)	MR (62%)	LR (30%)	NA[a]
FCM-based risk group	HR (5%)	6 (4%)	2 (1%)	0 (0%)	0 (0%)
	MR (38%)	3 (2%)	60 (35%)	1 (1%)	0 (0%)
	LR (59%)	0 (0%)	44 (26%)	49 (29%)	6 (4%) [a]

[a] Not applicable: Six patients could not be classified with molecular MRD analysis (no Ig/TCR marker with at least a quantitative range of 10^{-4}).

V.H.J. van der Velden et al., Unpublished results

Risk group definition



Current position of FCM in MRD diagnostics

1. FCM has proven to be useful for MRD detection, BUT:
   - does FCM measure the same as PCR?
   - can FCM replace PCR?
   - can FCM supplement PCR?
     (e.g. in cases without sensitive Ig/TCR targets)
2. FCM can easily become broadly available
   - no special laboratory facilities
   - high-tech FCM equipment (≥ 8 colors) for moderate costs

!!! ATTENTION FOR:

- standardization
- improvement of specificity and sensitivity (new markers)
- broadly accepted antibody protocols
- international guidelines for data acquisition and interpretation

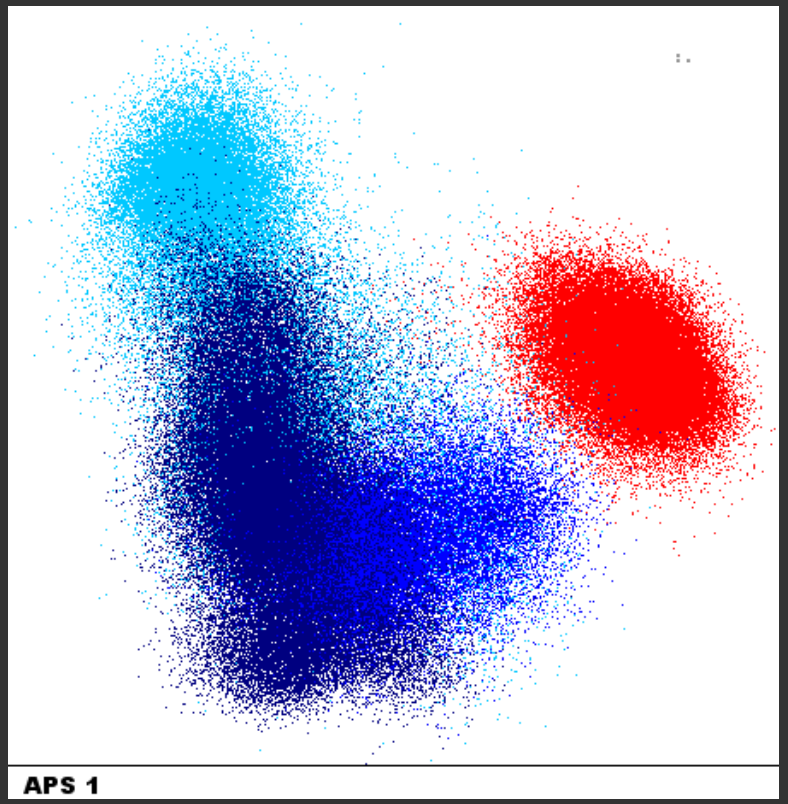
Aims in Flow cytometric MRD diagnostics

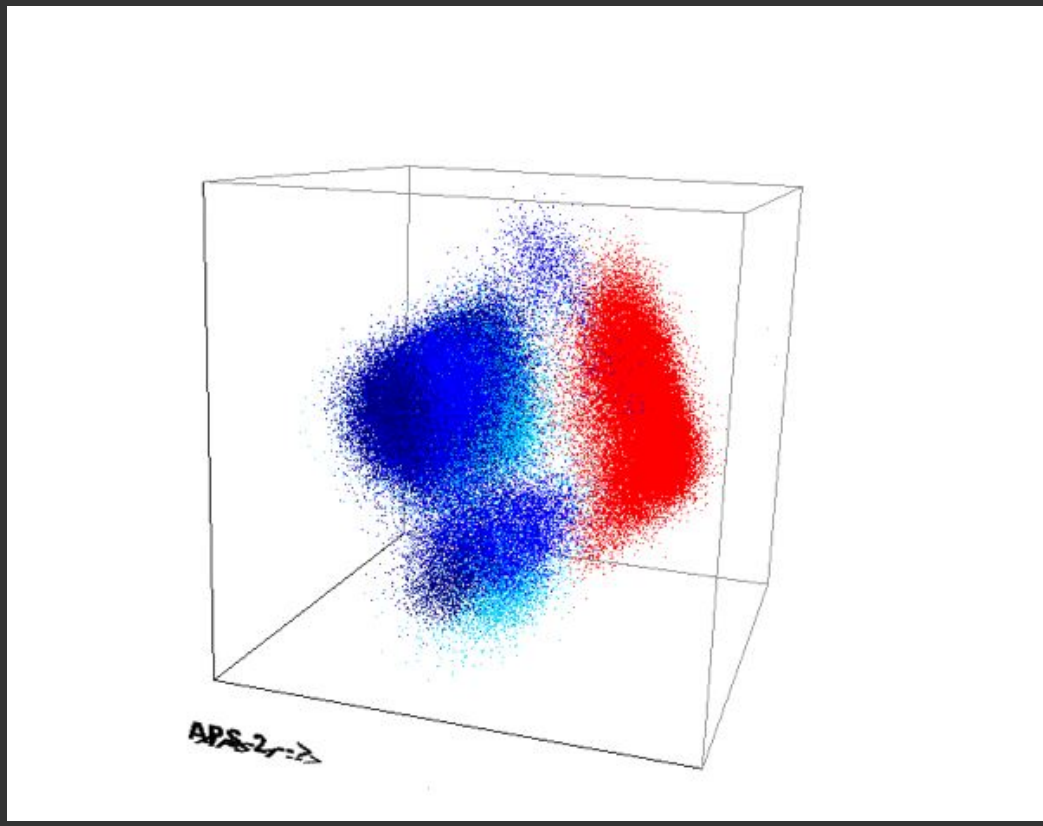
Fully standardized flow cytometric MRD detection !

1. Multicenter design, standardization, and clinical (protocol-related) evaluation of innovated flow cytometric MRD detection:
   - 8 colors: increased sensitivity
   - new markers (particularly fusion proteins/oncoproteins): increased specificity
   - new software (fast, easy, automated)

2. Evaluation of flow cytometric MRD detection in full parallel to Ig/TCR based MRD detection, using strict international guidelines for instrument settings, data acquirement, and data interpretation

BCP-ALL panel





Mix of 3 different regenerating B cell populations (Haematogones)

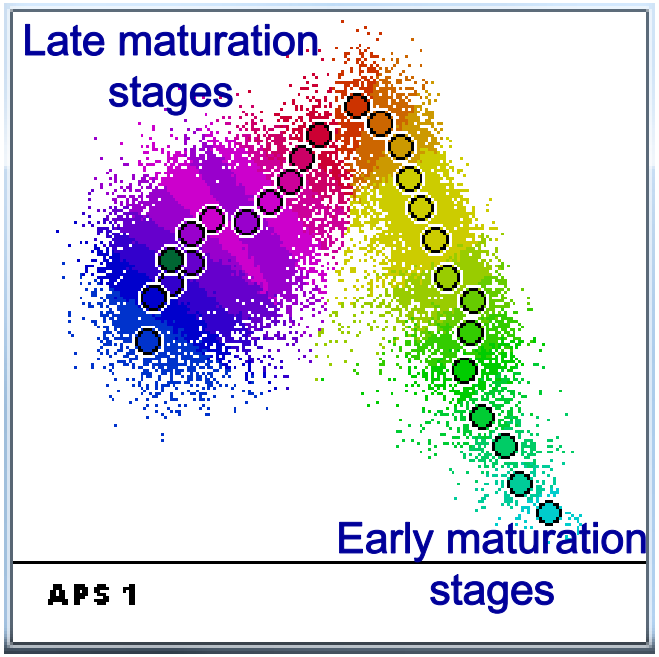
BCP-ALL blast cells

EuroFlow

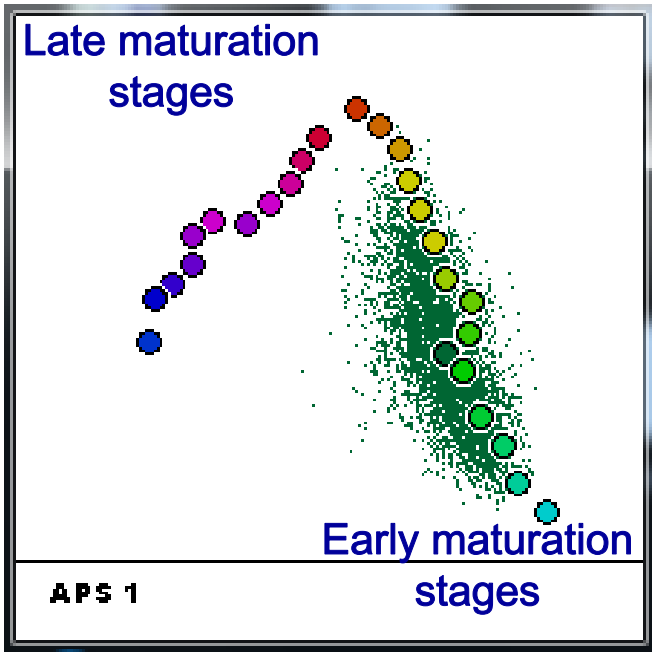
Responsible scientist: L Lhermitte

Precursor B-cell differentiation in normal vs regenerating bone marrow

Normal BM



Normal vs Regenerating BM



CD19 Gated B-cells (excluding PC)

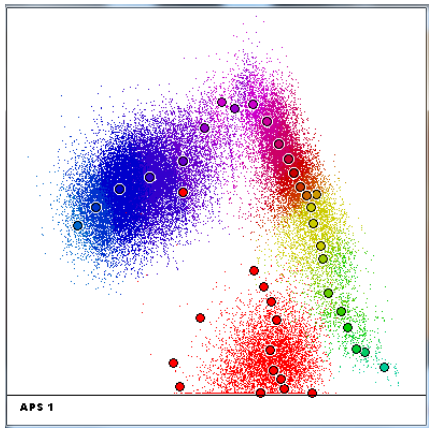
Responsible scientists: V.H.J. van der Velden and E. Mejstrikova

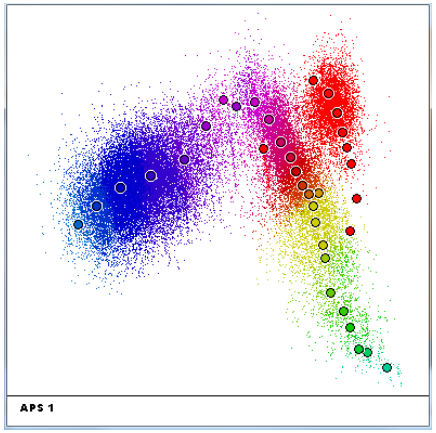
EuroFlow

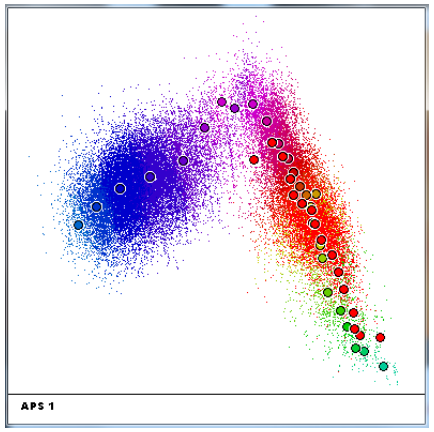
Four BCP-ALL cases vs normal precursor B-cells

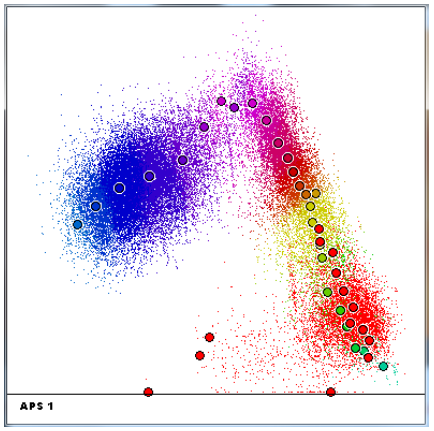
EuroFlow

APS view 1

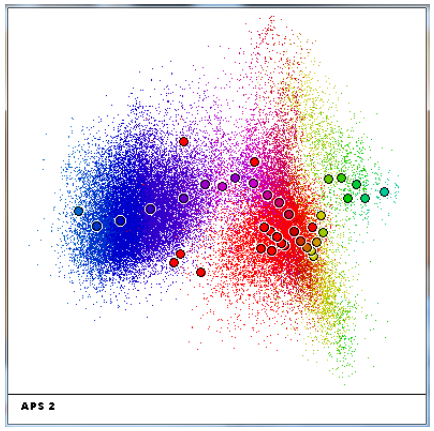


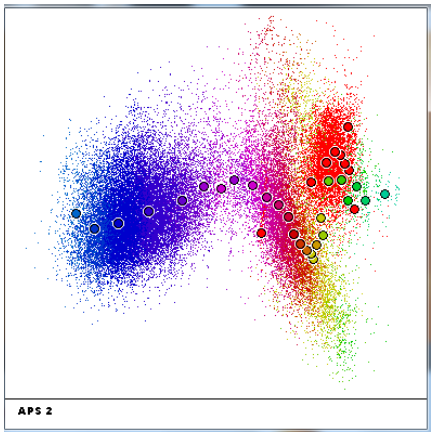


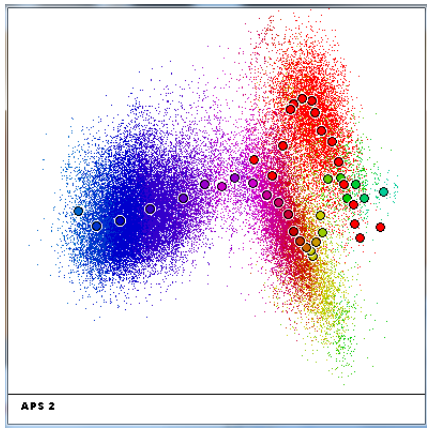


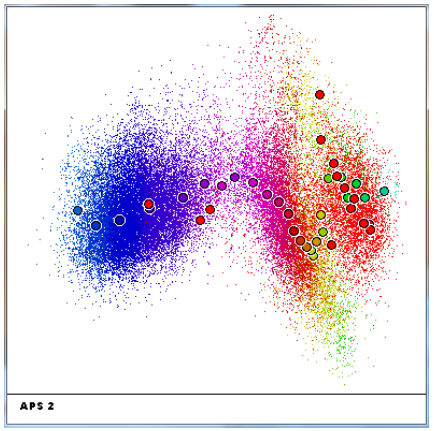


APS view 2









Case 1 Case 2 Case 3 Case 4

Responsible scientists: V.H.J. van der Velden and E. Mejstrikova

Designed by: A. Orfao & Q Lecrevisse

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Conclusions

1. PCR-based MRD diagnostics (IG/TCR genes of fusion genes) is currently the gold standard in many European ALL protocols
2. Differences in MRD value between protocols is mainly caused by application of different non-standardized MRD techniques, which also differ in sensitivity.
3. PCR-based MRD diagnostics can potentially be replaced by 8-color flow cytometry (Novel developments are required)
4. Standardization, regular Quality Control, and guidelines for data interpretation and data reporting are essential for international comparability of MRD results (within and between treatment protocols).

Collaborative networks on standardization & quality control are essential